kikkoman

Kit for the Rapid Detection of Total Coliforms

CheckLite CT150

(Coliforms Test)

For 150 assays

(Code 61328)

User's manual

- CheckLite CT150 is a rapid detection kit for the presence of total coliforms, using a sensitive bioluminometric assay for β-galactosidase in coliform bacteria.
- CheckLite CT150 is a more sensitive detection method than colorimetric assays
- CheckLite CT150 is a presence or absence test of coliform bacteria (not quantitative)

Principle of Measurement

CheckLite CT150 is an enzymatic presence-absence test for total coliforms based on the detection of β-galactosidase. Recommended media contain surfactant to propagate only gram-negative bacteria. The Detection Reagent CT150 contains D-luciferin-*O*-β-galactopyranoside (LuGal), a substrate derivative of both β-galactosidase and luciferase. Luciferin liberated from LuGal by β-galactosidase can be detected as luminescence using luciferase. The amount of bioluminescence produced in the reaction above is in direct proportion to the amount of β-galactosidase in the cultured and permeabilized coliform bacteria. A highly sensitive bioluminometric assay of β-galactosidase allows more rapid detection of coliforms than colorimetric assays.

Storage of Kit

Store the kit at 2-8°C in a refrigerator. Store the reconstituted detection reagent at 2-8°C, if it is to be used within the day and below -20°C if it is to be used within a week. When using the reconstituted and stored reagent, check the Value 1 and Value 2 (see Instructions for Use 3-5)) before use.

Composition of Kit

1. Detection Reagent CT150:
   5 green-labeled vials
   These contain D-luciferin-*O*-β-galactopyranoside (LuGal), recombinant firefly luciferase, ATP in lyophilized form.
2. Solvent Water for detection reagent CT150:
   6.3 ml x 5 pink-labeled vials
   These contain ultra-pure water for dissolving the detection reagent CT150.
3. Positive Control CT150:
   2 ml x 1 blue-labeled vial
   This contains β-galactosidase from *E. coli*.

The kit contains reagents for 150 assays.

Precautions

1. During operation, use laboratory gloves and a mask to avoid the contamination of β-galactosidase.
2. Static electricity on LumiTube or Luminometer may cause Lumitester to indicate abnormal values. To prevent static electricity, wipe the LumiTube and the Luminometer with a moistened towel.
3. Use anti-static electricity gloves (such as nitrile rubber) and avoid rubbing LumiTube with globes.
4. Electric noise from a microwave, a mixer and the like may cause Lumitester to indicate abnormal values. Turn off or remove those apparatus during the measurement.
5. Do not use an expired kit.（Expiration date is printed on the kit box）
6. Do not use components from different kit lots in an assay.
7. Recommended luminometers should be used to measure luminescence intensities.
8. Recommended medium should be used for cultivation of samples.
9. After the reconstitution of the detection reagent CT150, transfer it to LumiTubes immediately, and keep them at a room temperature (20-30°C) for 30 to 60 min.
10. If you need to use more than one vial of lyophilized reagent at a time, reconstitute the correct number of vials and mix all reagents before use.
11. Wipe the inside of the chamber cover of a luminometer with a paper towel moistened with 70-80 % ethanol to avoid contamination with luciferin, that may have spilt during a prior use of another CheckLite kit.
12. The kit detects β-galactosidase produced by coliforms. Since each coliform produces a different amount of the enzyme, this kit is not quantitative.
13. You cannot detect all the coliforms by 5 to 7 h culture. The longer the culture time is (up to 24 h), the more accurate results you can obtain. With overnight culture by this kit, you can detect almost all kinds of coliforms, which may not be detected on agar plates in the same culture time. Although in rare case, some coliforms are difficult to detect by this kit.

Instructions for Use

1. Materials Required but not Provided

1) Pro-media XM Broth (Elmex, Tokyo) or CT Medium (0.55% Nutrient Broth No.2 (Oxoid),

0.078% Tryptone (Difco), 0.39% NaCl, 39 mM K_2HPO_4, 0.1% Sodium Deoxycholate, 0.1 mM IPTG, pH 7.3)

2) Lumitester C-110 (Kikkoman Biochemifa Company, Code 61911)
3) LumiTube (Kikkoman Biochemifa Company, Code 61316)
4) Tube stand
5) Incubator
6) 20-100 μL pipetter, 200-1000 μL pipetter and disposable sterilized pipetter tips
7) Laboratory gloves (anti-static electricity)
8) 70-80% Ethanol
9) Autoclave or sterilizer

2. Sampling and Propagation

1) Pre-incubation of Test Media
   Before the test, pre-incubate test media (usually one more medium than test samples) at 35 to 37°C.
2) Sampling
   (a) Swab on Washed Surfaces
   Wet a swab using saline. Wipe washed surfaces carefully with it. Be careful not to contaminate the swab before use. Dip the swab into 2 mL of Pro-media XM broth or CT medium and carefully transfer the captured microorganisms into the media.
   (b) Swab on Dirty Surfaces
   In the case of swabbing dirty surfaces, a high blank value may prevent the rapid test. We recommend the following procedure. Dip the swab into a buffer (1 to 10 mL) and add an aliquot (around 0.2 mL) to 2 mL of medium for propagation. When using larger amount of aliquot, add the aliquot to ten times the amount of medium. Keep the remaining sample in a refrigerator for blank value measurement (see 4-2))
   (c) Food and Dairy
   Use directly or do an extraction with buffer such as phosphate buffer. Samples or extracts are to be inoculated into usually ten times the amount of medium. For example, 2 mL of milk or food extract is inoculated into 20 mL of Pro-media XM broth. For blank value measurement, the remaining samples or extracts should be kept in a refrigerator (see 4-2)). In the case of testing several kinds of food or dairy samples at one time, it is necessary to measure each blank value. That means more media and reagents are necessary for the number of blanks. Fresh food sometimes contains considerable amount of β-galactosidase, which could increase the blank value and make the assay less sensitive.
3) Propagation
   Incubate the test medium at 35 to 37°C for 5 to 24 h without shaking. Determine the incubation hours required by referring to Table1. For blank value measurement, do not forget to incubate extra media with no inoculation together with test media with sample inoculation.

Table1 Incubation time required for detecting coliforms★

Strain	Incubation time
Escherichia coli IAM12119	5 h
Citrobacter freundii JCM1657	7 h
Enterobacter cloacae IAM12349	6 h

★initial coliforms per 2 mL of test medium: about 10 colony forming units

3. Preparation of Reagent

About one hour before the end of propagation, prepare the detection reagent CT150 as follows. To check the reagent itself, prepare enough reagents to perform one more assay than the number of test cultures plus blank value measurements. One vial contains enough reagents for 30 assays

1) Dissolve: Pour the Solvent Water from the pink-labeled vial into the opened green-labeled vial (Detection Reagent CT 150). Stir the vial gently so as not to produce foam until the contents are completely dissolved.
2) Mix: If you dissolve more than one vial, mix all the reagents before use in a clean tube or bottle (Well washed one that is not contaminated with β-galactosidase) and mix. If Value 1 measured in step 3-5 is more than 800 RLU, the tube or bottle may be contaminated.
3) Transfer and Incubation: Prepare 30 LumiTubes for each bottle of dissolved Detection Reagent CT150. Transfer 200 μl of the reagent to each LumiTube. Take LumiTubes filled with the reagent for the assay (one more assay than the number of test cultures plus blank value measurements) and incubate 30 min to 1 h at room temperature (20-30°C) (the luminescence becomes stable during this period).
4) Storage of the Extra LumiTubes Filled with the Reagent: Keep in a refrigerator within one day or keep at a freezer (below –20°C) for 1 week. Do not store the thawed reagent again. Store the tube stand in a plastic bag to prevent contamination of β-galactosidase. When using any stored reagent, incubate 30 min to 1 h at room temperature after its temperature reaches room temperature, and then proceed to 3-5.
5) Check the Performance of Detection Reagent.
   (1) Wipe the inside of the chamber cover of the Lumitester carefully with 70-80 % ethanol to remove any luciferin, which may have spilled during a prior use of another CheckLite kit.
   (2) Measure the amount of bioluminescence (RLU: Relative Light Unit) of the incubated reagent in a LumiTube with a Lumitester (Value 1). Value 1 should be less than 800 RLU. Reduced

incubation time or contamination by β-galactosidase may cause a higher RLU.

(3) Take the LumiTube filled with the reagent and add 40 μL of the positive control (blue-labeled vial), and leave it at 20 to 37°C for 10 to 30 min (the luminescence is stable in this period).

(4) Measure the RLU with a Lumitester (Value 2). Check if the Value 2 shows at least 40 times higher value than Value 1. If not, this may be due to contamination of the reagent by β-galactosidase or the reagent may not be working well. Prepare new reagent and check again.

4. Assay Procedure

1) Mixing and Measurement: Add 40 μL of test culture to each LumiTube containing 0.2 mL of the detection reagent using a pipetter and mix well. Let the mixtures stand for 20 min at 35 to 37 °C, and measure the luminescence with the Lumitester. β-galactosidase extracted from coliforms in the test cultures causes a continuous increase in luminescence during the incubation. Incubation more than 20 min results in extra extraction, which leads to higher luminescence. On the other hand, the blank value remains stable with longer incubation. You can get a more clear result with longer incubation up to 40 min, if you have time. We recommend you to keep the remaining test cultures at 35 to 37 °C in the case of re-measurement (see 5).
2) Measurement of Blank Value: Take the un-inoculated medium for the blank value measurement and perform operation 4-1.Mix the medium with the reagent and measure luminescence in the same manner (Value 3). If the samples are swabs on dirty surfaces or food or dairy products, it is necessary to use the mixture of the samples or the extracts kept in the refrigerator (see 2-2) and the medium incubated at 35 to 37 °C in the same ratio as the propagated test cultures instead of the medium only.
3) Value 3, which is the blank value, is very important for the interpretation of results. It is usually less than 1,200 RLU. Higher blank value indicates contamination by β-galactosidase. But in the case of swabbing dirty surfaces, food or dairy samples (especially fresh food), blank values may exceed 1,200 RLU due to the existence of β-galactosidase. If Value 3 exceeds 5,000 RLU, it usually indicates the sample contains a considerable amount of β-galactosidase, which makes the assay less sensitive.

5. Interpretation of Results

The test sample is coliform positive when the luminescence value of the test culture is higher than 2 times the blank value (Value 3). If it is more than 1.5 times but less than 2 times, the sample may be coliform positive. In such cases, we recommend you to measure the test culture again with an extra hour of incubation, which may make the results more definitive. If it is less than 1.5 times, the test sample is coliforms negative.

6. References

Masuda-Nishimura, I. et al. (2000) Lett. Appl. Microbiol. 30, 130-135

FOR SAFETY USE

Pay attention to the points listed below for safe operation of this kit.

1. CheckLite CT150 is not recommended or intended for the diagnosis of disease in humans or animals.
2. This kit is designed for use by personnel familiar with appropriate aseptic techniques for the isolation and identification of microorganisms.
3. Don't swallow or contact the reagents supplied with this kit with skin or eyes. In case of swallowing or contact with the skin or eyes, rinse immediately with plenty of water and seek medical advice.
4. Store and discard this kit with care so that you do not contaminate food or other products with the reagents and materials supplied with the kit.
5. Be careful not to spill the propagated sample for biohazard reasons.
6. Keep this kit away from children and infants.

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